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A T vector with very low background levels

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▼T vectors (linearized plasmids containing single 5′ T overhangs) allow the direct and efficient cloning of PCR products by taking advantage of the terminal transferase activity of *Taq* polymerase that, under standard PCR conditions, leads to the addition of a single nucleotide (usually dATP) to the 3′ ends of PCR products. To facilitate vector preparation, several plasmid and phagemid DNAs have been created containing two adjacent non-identical restrictions sites for *Xcm*I (Ref. 1, 2, 3, 4, 5, 6). Digestion of such vectors with *Xcm*I generates a linear DNA molecule with single T overhangs at both 3′ ends. However, a problem with the use of these vectors is that the background of non-recombinants is often unacceptably high (Ref. 1, 2, 4). This has been suggested to be the result of partial digestion of the plasmid DNA generating a low level of singly cut plasmid molecules that can efficiently self-ligate (Ref. 2). Partial digestion often occurs because: (1) *Xcm*I appears to digest DNA less efficiently than many other restriction enzymes; and (2) the enzyme is relatively expensive, such that the number of units/μg DNA used in the digestion reaction is often kept to a minimum. To counteract this, various strategies have been reported for reducing the background associated with these vectors, including dephosphorylation of the digested vector DNA (Ref. 1) and inclusion of the *Xcm*I enzyme in the ligation reaction; such strategies either reduce cloning efficiency or are expensive. A simpler method would be to gel-purify the fully digested vector DNA from the partially digested DNA, directly after the digestion reaction. However, with all current *Xcm*I-based T vectors this is not possible because partially digested and fully digested vector DNA are very similar in size and cannot be separated by conventional agarose gel electrophoresis.

To resolve this problem we have constructed a plasmid T-vector, pT-NOT, which contains a 450 bp stuffer fragment from the vector pEF-BOS (Ref. 7) inserted between two appropriate *Xcm*I sites (Figure 1). After *Xcm*I digestion, partially digested DNA, which still contains the stuffer fragment, can be easily separated from fully digested DNA by standard agarose gel electrophoresis because of the 450 bp difference in size. In PCR product cloning experiments with this vector, there is virtually no background and recombinants containing the correct insert account for between 90% and 100% of the bacteria colonies tested. The finding that this modification effectively eradicates the background problem indicates that the background seen with other *Xcm*I-based T-vectors is probably not the result of contaminating nucleases in the *Xcm*I enzyme preparation, as has been previously postulated (Ref. 1). A further improvement to this vector is inclusion of flanking *Not*I sites. As *Not*I is a rare eight-base cutter, most inserts can simply be excised from the vector by digestion with *Not*I alone. The plasmid is available upon request.

References

1 Cha, J., Bishai, W. and Chandrsegaran, S. (1993) *Gene* 136, 369–370.
2 Harrison, J., Molloy, P.L. and Clark, S.J. (1994) *Anal. Biochem.* 216, 235–236.
3 Kovalic, D., Kwak, J.H. and Weisblum, B. (1991) *Nucleic Acids Res.* 19, 4560.
4 Mead, D.A. *et al.* (1991) *Biotechnology (NY)* 9, 657–663.
5 Schutte, B.C., Ranade, K., Pruessner, J. and Dracopoli, N. (1997) *Biotechniques* 22, 40–44.
6 Testori, A., Listowsky, I. and Sollitti, P. (1994) *Gene* 143, 151–152.
7 Mizushima, S. and Nagata, S. (1990) *Nucleic Acids Res.* 18, 5322.

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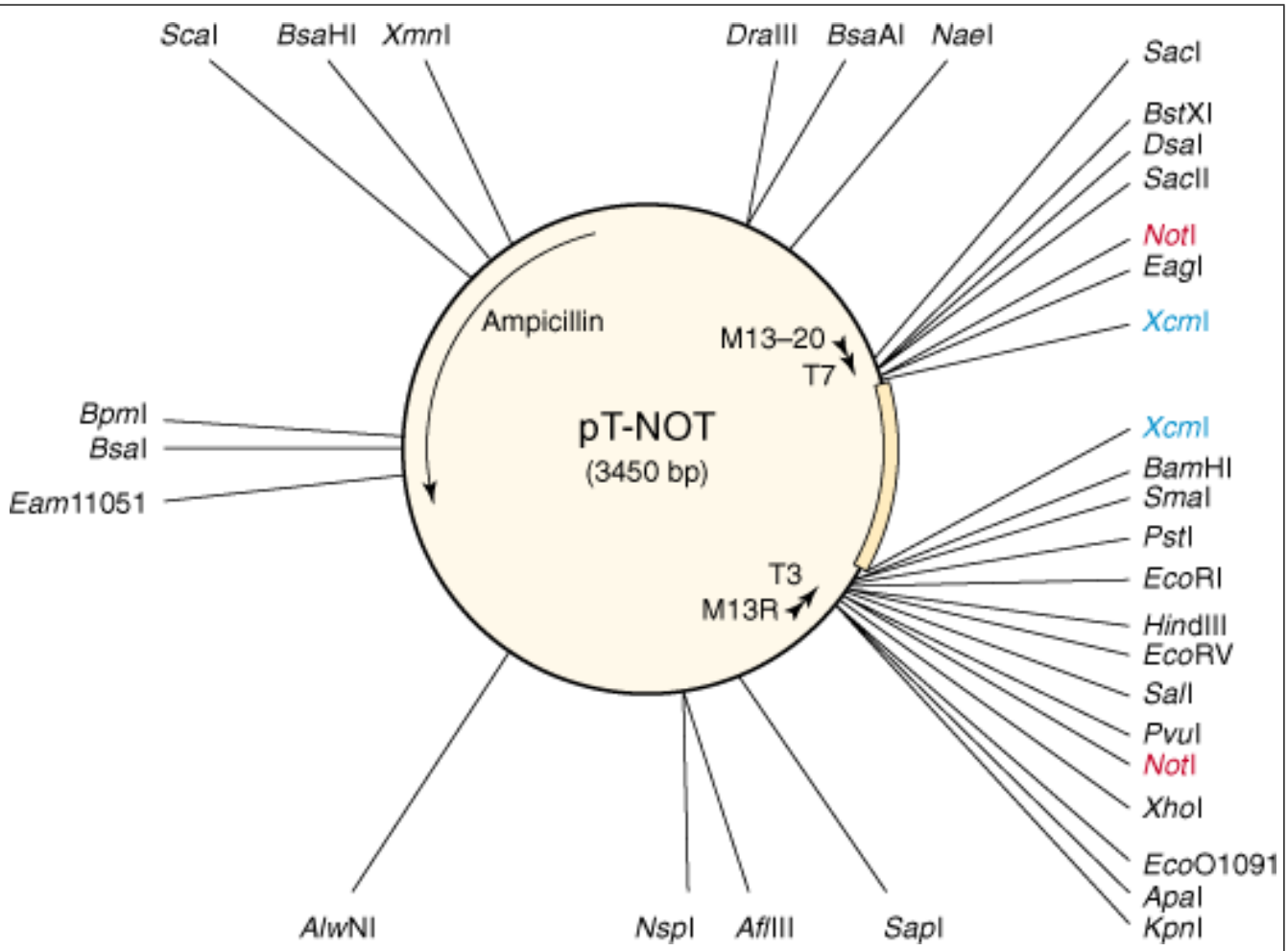

FIGURE 1. The pT-NOT plasmid. The yellow box represents the stuffer fragment. Restriction sites in the polylinker as well as unique sites in the rest of the vector are shown. *Xcm*I sites are labelled blue and flanking *Not*I sites are labelled red. Locations of M13-20, M13 reverse, T3 and T7 primers are indicated. The plasmid backbone is pBluescript (KS+).